BRIEFER ARTICLES

CHLOROFORM AS A PARAFFIN SOLVENT IN THE IMBEDDING PROCESS

In a recent number of this journal LAND[1] describes an improved method of replacing the paraffin solvent with paraffin, in which he calls attention to certain difficulties encountered in the use of xylol as a solvent, and offers some suggestions by which these difficulties may be overcome.

The writer is somewhat surprised to find that xylol is so extensively used as a paraffin solvent in the process of infiltration, and for this reason he is tempted to describe the method that he has used for a number of years. In the first place, judging from the writer's experience, the best way, or at least one of the best ways, to avoid the difficulties mentioned by LAND in the use of xylol is to do away with the xylol altogether. Nineteen years ago the writer discarded the use of xylol. In its place he used chloroform, and he has been convinced that this solvent is superior to xylol. His method is as follows: We assume that the material has been carefully and thoroughly dehydrated by passing through the grades of alcohol, beginning with very low percentages, depending upon the character of the tissue. In dehydrating very soft objects one may begin by adding a few drops of alcohol at a time until a strength of 10 or 15 per cent has been reached. I have found this a safe thing to do in the case of objects like the seaweed *Champia parvula*. Some objects, however, may be placed directly from water into 20 per cent alcohol. It does not seem that standing overnight in any of the weaker grades of alcohol injures the tissues in the least.

After the specimens have been thoroughly dehydrated (I refer chiefly to material for morphological and cytological purposes), they are placed in a mixture of equal parts of absolute alcohol and chloroform, where they should remain for 2–3 hours or longer. When thrown into this fluid the specimens float, but in a short time they sink to the bottom of the vessel. No injury results if the specimen be allowed to remain in the chloroform and alcohol overnight or longer. Next the specimens are placed in pure chloroform, where they remain for 2–12 hours, depending upon the size of the specimens, the nature of the tissue, and to some

[1] LAND, W. J. G., Microtechnical methods. BOT. GAZ. **59**:397–401. 1915.

extent upon the composition of the fixing or killing reagent. Generally the material is left in the pure chloroform 2 hours, or until it sinks to the bottom of the vessel. Material such as root tips or lily anthers fixed in chromo-osmic-acetic acid or chromo-osmic acid will sink in pure chloroform within 2 or 2.5 hours, but if chromo-acetic acid, absolute alcohol, or reagents that do not contain osmic acid are used as killing reagents, the specimens sink slowly or not at all. In the case of material fixed in chromo-acetic acid, for example, the specimens are left in the pure chloroform overnight or longer if necessary.

The specimens are now changed to a fresh quantity of pure chloroform, and shavings of paraffin (melting point 42–45°) are added until at room temperature no more paraffin will dissolve. It will be seen that the paraffin floats at the surface of the fluid, while the specimens, in case they have sunk, are at the bottom. The chloroform surrounding the objects becomes gradually saturated, therefore, at room temperature. If the objects have not sunk in the chloroform, they do so gradually as the paraffin is dissolved. The degree of saturation of the solution may be increased slowly by adding paraffin a little at a time, but the writer has not found any special care necessary. When the chloroform is saturated at room temperature, the vessel is placed upon the paraffin oven and a little more paraffin is added if desired. The vessel, which is still closed with a stopper, remains on the oven 2–12 hours. The contents are now poured out into an open dish (usually a small porcelain dish) and covered by only a slip of paper to keep out dust, and this dish remains upon the oven until so much chloroform evaporates that the paraffin congeals slightly at the edge of the dish or over the whole surface. This requires usually one night. The dish is then placed inside the oven and allowed to remain until all the chloroform has evaporated, as determined by taste. The specimens are now transferred to melted paraffin of 52–55° melting point, or that of a higher melting point if necessary, in which they remain 10 minutes to 2 hours or longer before imbedding. They are then imbedded in this or similar paraffin.

Although chloroform is expensive, this is not necessarily a costly process. The paraffin (45° melting point) used in making the chloroform-paraffin solution may be used over again two or three times, and 8 cc. of chloroform in each vessel is sufficient for a quantity equal to 10 or 15 root tips of onion or lily anthers.

It is understood, of course, that chloroform should be kept out of direct sunlight, preferably in a dark place.—D. M. MOTTIER, *Indiana University, Bloomington, Ind.*

In the Hull Botanical Laboratory, after a long series of rigid comparative tests of the various paraffin solvents in general use, it was found that xylol when carefully used gave uniformly better results than any other solvent. Cedar oil was rejected because it is almost if not quite impossible to eliminate the oil in the final stages of imbedding. It is true that hard material cuts somewhat better after cedar oil, but the same end may be attained in a far better way by soaking the imbedded material in water.

Chloroform was abandoned because in transferring from alcohol to chloroform it was found that, even when a much closer series than the one recommended by Professor MOTTIER is used, some plasmolysis results. Also we have found that paraffin does not seem to penetrate the tissues as readily after chloroform as after xylol. These results should be expected when we remember that the specific density of chloroform is nearly twice that of either alcohol, xylol, or paraffin. In STRASBURGER'S laboratory chloroform was practically abandoned for xylol about 15 years ago. We have in this laboratory preparations of root tips as well as of *Lilium* anthers showing reduction division, which, from the maker's name, we assume were made exactly as described by Professor MOTTIER. These preparations are certainly inferior to those in which xylol was used as a solvent. Dr. L. W. SHARP, one of the most successful workers in the peculiarly difficult field of modern cytological technique, always uses xylol as a solvent.

If all stages in cytological technique received equal care, published results would undoubtedly be in closer accord than they are at present.—W. J. G. LAND, *University of Chicago.*

BESSEYOSPHAERA, A NEW GENUS OF THE VOLVOCACEAE

Two new species of the Volvocaceae were described by POWERS[2] without names or assignments to taxonomic positions. They were designated "first form of *Volvox*" and "second form of *Volvox*." The former was subsequently further described and named *Volvox spermatosphaera*.[3] The "second form" is intermediate between *Pleodorina* and *Volvox* in the scale of differentiation, and its assignment to either genus would involve so great an extension of the conception of the

[2] POWERS, J. H., New forms of *Volvox*. Trans. Amer. Mic. Soc. **27**:123–149. 1907.

[3] ———, Further studies in *Volvox*. Trans. Amer. Mic. Soc. **28**:141–175. 1908.